CHAPTER 1

The immunobiological effects of IL-2 *in vivo*

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IL-2 THERAPY IN CANCER

The first report on IL-2 administration to human cancer patients dates back to 1983 (1) and the first clinical responses to IL-2 therapy were reported by Rosenberg in 1985 (2). Since then, interleukin-2 (IL-2) therapy has been tested as a new form of immunotherapy in cancer patients (2-5). It turned out that IL-2 is active in mainly just two types of cancer: melanoma and renal cell carcinoma (RCC). In addition, only twenty percent of patients suffering from these types of cancer respond to IL-2 therapy, with mostly partial remissions. A major clinical draw-back of IL-2 therapy is its toxicity. The most life threatening form of toxicity is the capillary leak syndrome. Other manifestations are fever, headaches, dyspnoea, and hypotension. Toxicity is dose-related in the case of intravenous (iv) administration. Subcutaneous (sc) administration of rIL-2 has been reported to be less toxic, also at high doses, while response rates are comparable with bolus or continuous intravenous infusion (6-8).

Various doses of Il-2, as well as various times and ways of administration have been tried out to improve IL-2 therapy, but none of these outweigh the others in terms of tumour response. Combinations of IL-2 therapy with infusion of cells or with other cytokines have been tried and are still under investigation, although most of these do not appear to be very promising. So, it is still not clear whether IL-2 administration in combination with LAK cells (Lymphokine Activated Killer cells, mainly of the NK cell phenotype) does enhance response and survival rates in RCC and melanoma or not (9-12). The reported better therapeutic efficacy of the combination of IL-2 plus TIL (Tumour Infiltrating Lymphocytes, mainly of the T cell phenotype) has also still to be confirmed (13-15). Of the combinations of IL-2 plus interferons or TNFα (tumour necrosis factor-α) (8,16-20), only the combination of IL-2 plus IFNα (Interferon-α) has been compared to IL-2 alone in similar settings (21,22). In contrast to results found in animals (23,24), there seems to be no synergistic effect of IFNα on the immunomodulatory and anti-tumour effects of IL-2 in patients.

So, apart from some down modulatory effect on IL-2 related toxicity (sc rIL-2 treatment), variations of IL-2 therapy or combinations of IL-2 with other agents do not enhance response rates. Apparently, IL-2 alone is capable of inducing the biological effects associated with responses seen thus far.

Currently, investigators try to introduce cytokine genes in TIL or tumour cells (10,25-27). TIL migrate specifically to the site of the tumour (28). It is hypothesized that, by transferring cytokine gene transformed TIL to patients, high levels of cytokines will be produced at the site of the tumour, and not in the rest of the body, preventing systemic toxicity. Transfection of tumour cells with cytokine genes or genes encoding for molecules participating in antigen presentation might transform tumour cells to professional antigen presenting cells, which can be used as a vaccine (29,30).

IL-2 was discovered as a T cell growth factor (TCGF) (31) but further studies showed that IL-2 stimulates a large array of cells, including T cells and NK cells (32). Concurrently, IL-2 induces directly and/or indirectly a broad scala of biological effects. Until now it is not known which of these effects contribute to the reported anti-tumour activity and which to toxic side effects. Such a lack of knowledge poses a number of problems. For instance it can be argued that experiments to avoid (toxic) side effects should be approached with caution, since the biological processes inducing toxicity might be responsible also for the anti-tumour activity. Indeed, correlations between toxic side

effects (capillary leak syndrome) and response have been reported (33), suggesting common biological mechanisms for response and toxicity. Another problem is that it is not known which biological effects should be monitored (besides the clinical effects), making it difficult to predict outcome of therapy or to determine whether it is clear that a newly designed variation on IL-2 therapy is better or not.

In conclusion, right interpretation of the various biological effects of IL-2 is important for, firstly, the identification of parameters which reflect the (biological) efficacy of IL-2 therapy. Secondly, such a knowledge might induce the development of treatments possibly preventing toxic side effects without affecting the efficacy of anti-tumour activity. Thirdly, and most importantly, a better knowledge of IL-2 biology might lead to improved protocols of IL-2 therapy enabling higher response rates and possibly also the application of IL-2 in more tumour types. The present review will give a survey of current knowledge of the biological effects induced by IL-2 in human cancer patients. How some of the goals that are set above can be reached is also indicated.

EFFECTS OF IL-2 ON LEUCOCYTE NUMBERS

In vivo IL-2 induces changes in numbers and phenotypes of various types of leucocytes. During administration of IL-2, lymphopenia is induced, whereas during days of rest (no IL-2 administration) the number of lymphocytes increases steeply (rebound lymphocytosis). Probably, lymphocytes adhere to a higher extent to endothelia and leave the circulation during IL-2 administration (increased efflux), whereas the influx of lymphocytes from the lymphoid organs and/or tissues is lowered. Outside the circulation, lymphocytes might become (further) activated and start to proliferate. They reappear subsequently in the circulation during the days of rest. Both T cells and NK cells follow this pattern, although the number of NK cells is the first to decrease during bolus IL-2 administration (34).

In addition to these changes in lymphocyte numbers, IL-2 induces eosinophilia starting after one week of therapy. In contrast to the pattern of changes in peripheral blood lymphocyte number, the absolute number of eosinophils increase during IL-2 administration and decrease during days of rest (unpublished observations). It has been shown that IL-2 therapy does induce IL-5 production, whereas no or almost no GM-CSF production is triggered (35). This suggests that the eosinophilia is the result of an elevated IL-5 level, which induces release of eosinophils from the bone marrow and also stimulates their further development (36). The kinetics of IL-5 in the blood show an increase during IL-2 administration and a decrease during the rest period (35). This corresponds with the observed pattern of eosinophil changes. Besides this indirect stimulation of eosinophils via IL-5, IL-2 might also play a direct role on eosinophil differentiation and activation, since *in vivo* activated eosinophils express the low affinity receptor for IL-2, CD25, as has been shown in hypereosinophilic patients (37). Eosinophils taken from patients during IL-2 therapy showed an increase in both direct as well as antibody dependent cytotoxicity against allogenic tumour cells (38).

Only few reports deal with changes in monocyte and neutrophil numbers during IL-2 therapy. We have noted an increase in the number of monocytes during the first two weeks of rIL-2 therapy, after which the number slowly decreased again (own unpublished results). Increases in the first 2 weeks were seen during both lymphopenia and lymphocyte rebound. Another report showed a correlation between response and decrease in number of monocytes after one cycle of continuous IL-2 therapy (39).

PHENOTYPICAL AND FUNCTIONAL CHANGES OF PBL

The NK cell population (identified by expression of the surface markers CD16 and/or CD56, without CD3 expression) is the most predominantly stimulated peripheral blood lymphocyte (PBL) subpopulation. Both relative and absolute numbers of NK cells increase with each cycle (40-47). At the end of therapy most NK cells express high levels of the CD56 antigen. These $CD56^{bright}$ cells express CD16 to a much lower extent than $CD56^{dim}$ cells. It has been suggested that these $CD56^{bright}CD16^{-/dim}$ cells are NK precursors. These cells show higher proliferation rates to IL-2 stimulation, equal TNFα but lower IFNγ production, and higher cytotoxic capacity, as compared to $CD16bright^{+}CD56^{-/dim}$ NK cells (48). However, recently it has been suggested that $CD56^{-/dim}$ cells might also be NK precursor cells (49).

During IL-2 therapy, NK cells show increased expression of the activation markers HLA-DR and CD38. Mainly the $CD56^{bright}$ cells appeared to express these markers (44). In addition, the expression of the intermediate affinity receptor for IL-2, IL-2Rβ, increased more than threefold on NK cells during IL-2 therapy without concomitant expression of the low affinity receptor IL-2Rα (CD25) (50,51). The expression of the IL-2Rβ alone is sufficient for NK cells to respond to IL-2 *in vitro* although the participation of a third subunit, IL-2Rγ, might also be involved in this response. The phenotypical changes of NK cells are accompanied by increases in NK and LAK activity (42,46,47,52). It has been reported that also the precursor frequency of LAK cells increased during IL-2 therapy (53).

In contrast to the well-known findings concerning IL-2 induced NK cell activation, there are only few reports dealing with T cell activation during IL-2 therapy (44,54,55). Only in the first week of IL-2 therapy, peripheral blood T cells seem to be activated as assessed by the expression of HLA-DR and CD38 (44). Subsequently, the numbers of HLA-DR and CD38 expressing T cells decrease again, especially within the $CD8^{+}$ T cell subpopulation. *In vitro* stimulation of PBL with IL-2 induces CD25 expression on 20-30% of all T cells, including $CD8^{+}$ T cells, and on 50% of all NK cells (unpublished results). In contrast, *in vivo*, during administration of IL-2, CD25 becomes expressed mainly on $CD4^{+}$ T cells but not on NK cells and only minimally on CD8 T cells (44,51).

Several groups have shown that during IL-2 therapy peripheral blood T cells have a decreased proliferative response to various stimuli. The *in vitro* proliferation rates of cells taken during IL-2 therapy in response to PHA, ConA, PWM, CD3 MAb, CD3 MAb + IL-2, PWM, tetanus toxoid and alloantigen were lower than the values obtained from cells taken before therapy (44,56-61). In addition, the *in vitro* expression of CD25 and HLA-DR on T cells in response to CD3 MAb is diminished on cultured cells obtained from PBMNC isolated during IL-2 therapy as compared to similar cultures of pre-therapy cells (44,59). The observed decrease in proliferative responses to the T cell specific stimuli (CD3 MAb, alloantigen, tetanus toxoid) is not due a relative decrease in T cell percentages in the cultures. In contrast to the decreased *in vitro* proliferative response to tetanus toxoid, the *in vivo* response to tetanus toxoid, as assessed by Ig production after an *in vivo* tetanus toxoid boost during IL-2 therapy, was enhanced (58). In another report, it was demonstrated that lymphocytes obtained during IL-2 therapy from responding patients showed higher non-MHC restricted cytolytic capacity in response to CD3 MAb than lymphocytes obtained from non-responding patients (61). This induction of anergy, might also occur at the site of the tumour, since TIL obtained

during IL-2 therapy showed a decreased proliferative response to IL-2 and decreased cytolytic activity against autologous tumour cells compared to TIL obtained before therapy (62,63).

Pilot experiments in our lab showed that the addition of CD28 MAb to cultures of PBL obtained during IL-2 therapy restored or even enhanced the proliferative response to CD3 MAb. This means either that peripheral T cells obtained during IL-2 therapy have a higher threshold to get activated, or that the B7-CD28 interaction of PBL obtained during IL-2 therapy is hindered.

The proliferative response to IL-2 and the NK and LAK activity of PBL taken during the lymphopenic phase of the first course (i.e. during IL-2 administration) is decreased. The decreased response to IL-2 is due to nonresponsive T cells, whereas NK cells are still able to respond (51). The peak in proliferative response to IL-2 of cells obtained after *in vivo* IL-2 administration is shifted from six days to three days of culture (56,57,64). The shift to a more rapid response to IL-2 might be due to the *in vivo* induction of IL-2R on the cells. Prolonged IL-2 therapy induces an increase in the proliferative response to IL-2 and in the *in vitro* NK and LAK activity, both in cells taken during the lymphophenic phase as well as during the phase of rebound lymphocytosis (41,56,57,64). LAK activity and fresh tumour lysis, however, are only detectable in the presence of IL-2 (64).

Whereas Weil-Hillman *et al.* (59) reported that killing of a human colon carcinoma cell line by retargeted T cells obtained after 4 weeks of therapy was decreased, Yoshino *et al.* (54) were able to demonstrate that after one week of IL-2 therapy, reversed ADCC killing of K562 and Daudi cells by T cells redirected against these targets by CD3 mAb was significantly enhanced compared to pretherapy activity. This killing appeared to be predominantly mediated by CD8 T cells and was enhanced by the addition of IL-2. Whether this discrepancy is due to difference in time point of analysis or difference in targets remains to be determined.

The activation of polymorph nuclear cells (PMN) has been studied by analyzing plasma levels of elastase α_1/antitrypsin complexes and lactoferrin, which are produced by activated PMN and by assessing *in vitro* salicylate oxidation. It could be demonstrated that PMN become activated during IL-2 therapy (65,66). Peak values in elastase α_1/antitrypsin and lactoferrin were observed 4-6 hours after bolus IL-2. This activation is probably an indirect effect resulting from the rise in serum TNFα (see below). PMN activation was parallelled by activation of the complement cascade as demonstrated by a rise in C3a (65,67). PMN acquire a chemotactic defect during IL-2 therapy, which might explain the high frequency of bacterial infections in patients receiving high dose IL-2 (68-70). *In vitro*, serum of IL-2 treated patients induced a deficiency in chemotaxy of neutrophils isolated from healthy donors (71). Addition of anti-TNFα antibodies to these assays induced dose-dependent reduction of this neutrophil chemotaxis deficiency, suggesting that TNFα is responsible for this defect.

CYTOKINE CASCADE

The production of a large number of other (secondary) cytokines is induced during IL-2 therapy. TNFα is probably the first secondary cytokine which is induced since the level of TNFα rises immediately within minutes after the start of rIL-2 therapy. In one study, administering $6x10^6$ U/m^2 of rIL2 (Roche) via iv bolus infusion, it was indicated that TNFα levels follow a biphasic pattern with peaks at 15 min and 4 hours after IL-2

administration (34,72). In another study, administering much higher doses of IL-2 ($6x10^5$ IU/kg of rIL-2 (Cetus) via iv bolus infusion), it was reported that there was one peak 2 hours after infusion (73). Peak levels of TNFα in the latter study reached values of 1 mg/ml, whereas in the study using moderate doses of IL-2, peak levels were less than 30 pg/ml. After reaching peak levels, the TNFα concentrations decrease rapidly again. The sources of TNFα production may be numerous, but since the increases in TNFα production are so rapid, the producing cells should be IL-2R positive and presumably contain cytoplasmically stored TNFα. A likely candidate is the NK cell population which consistently expresses p75 IL-2R and is able to respond via this receptor alone (51).

The rise in TNFα is followed by a rise in IFNγ with peak values on day 3 after bolus IL-2 (72). IFNγ is mainly produced by T and NK cells and induces activation of monocytes. Indeed, following the IFNγ peak there are peaks in the serum levels of neopterin, a pteridine selectively released by IFNγ stimulated monocytes and macrophages. *In vitro* experiments showed that neopterin is induced by IFNγ but not by IL-2 (72). It is unclear whether IFNγ is induced directly by IL-2 (74) or via other cytokines like TNFα and IL-1. IL-2 therapy induces also IL-1α, IL-1β and IL-6 (73,75,76), which also might be an indirect effect resulting from elevated TNFα as demonstrated for IL-6 after TNF administration (77).

Northern blot analysis showed that PBMC obtained after IL-2 administration expressed mRNA for TNFα, IL-6, very low levels of mRNA for IL-1β and not for TNFβ and GM-CSF (76). The mRNA of these latter cytokines, however, could be induced in these same cells by *in vitro* incubation with IL-2. No IL-3 or GM-CSF elevations were found in other studies using ELISA or RIA (35,78,79). Using RT-PCR, however, it was possible to detect the induction of mRNA encoding for IL-3, M-CSF and GM-CSF in mononuclear cells during IL-2 therapy (79). So, low levels of CSF, undetectable by immuno-assays or northern blot analysis might still be produced.

TNF might play an important role in the IL-2 therapy induced anti-tumour activity. Indeed, it has been reported that sustained elevated TNF levels during IL-2 therapy correlate with clinical response (33). However, the study of Fraker *et al.* (80) shows that αTNFα antibodies reduced TNFα levels without affecting responses in mice. Similarly, administration of sTNF-receptors during IL-2 therapy in mice also reduced TNF levels but did not affect clinical responses either.

Still, TNF might be important in direct anti-tumour activity. High dose TNFα perfusion in patients with melanoma or sarcoma in the extremities has been shown to induce durable responses in about 90% of treated patients (81,82). This anti-tumour effect is probably due to a specific TNFα mediated destruction of the tumour vasculature, whereas the vasculature of normal tissue remains largely undamaged.

Comparisons of the various, in the literature reported, cytokine profiles are complicated by the types and sensitivities of the various assays used, by the variations in time points of analysis and by the presence of competitive soluble cytokine receptors in the assessed sera samples, which might or might not interfere with the assay. For instance, IL-2 therapy induced an increase in soluble TNF-R1 and TNF-R2 levels, in which maximum induced sTNF-R levels correlated with maximum induced TNF levels (83).

Patients with solid tumours, including metastatic RCC, have higher levels of sIL-2R than healthy persons. It is assumed that high levels of sIL-2R reflect activation of the

immunesystem, but it is unclear whether this is also true for cancer patients or not. Nevertheless, during the first week of IL-2 therapy, the levels of sIL-2R in plasma increase more than tenfold. The concentration of sIL-2R remains at this high level during the following weeks of therapy, thereby parallelling CD25 expression on T cells during therapy (44). sIL-2R levels increase during the lymphopenic phase and decrease during rebound (84). sCD8, which is released after $CD8^+$ T cell activation, is not elevated in RCC patients, but increases more than 2 times during the first week of IL-2 therapy after which sCD8 levels return to pretherapy values again. This pattern coincides with the kinetic pattern of HLA-DR expression on CD8 T cells, suggesting that sCD8 is produced by activated peripheral blood CD8 T cells during IL-2 therapy. Martens *et al.* showed that the relative increase of sCD8 during the first week of IL-2 therapy correlates with clinical response (85).

THE CAPILLARY LEAK SYNDROME

Endogenously produced TNFα is thought to be the main inducer of toxic side effects seen with IL-2 therapy (86). One of the major problems associated with IL-2 therapy is the capillary leak syndrome (cls) (87). Administration of dexamethasone, an inhibitor of TNF mRNA production and translation, during IL-2 therapy decreased TNF levels and reduced toxicity (88). In addition, Fraker *et al.*, (80) and Edwards *et al.* (89) showed that αTNF antibodies diminished the toxic effects including cls of IL-2 in mice and rats, without affecting anti-tumour activity. Antibodies against IFNγ had no effect. Results of experiments of Cotran *et al.* and Horvath *et al.* (90,91) suggested that TNFα might mediate the increased permeability of endothelial cells (EC) directly, and that this caused cls directly. However, Mier *et al* showed that IL-2 induced EC permeability is a cell dependent process (92,93), since only addition of IL-2 activated PBMC (LAK cells) resulted in increased EC permeability. Since EC are susceptible to LAK mediated cytotoxy, LAK cell mediated injury of EC might be the actual cause of the cls. This was confirmed *in vivo* by depleting NK cells in mice with anti asialo-GM-1 (94) or anti-NK MAb NK1.1 (95) during IL-2 therapy, which reduced IL-2 induced toxicity, without affecting anti-tumour activity. Depletion of either Lyt-2+ or L3T4+ T cells did not reduce toxicity but greatly reduced the anti-tumour activity (95). Surprisingly, EC pretreated for 18 hours with TNFα, IFNγ or IL-1β were less susceptible to LAK cell mediated lysis and had a decreased permeability compared to unprimed EC, despite the fact that TNFα increases the number of adherent PBMC (92). Indeed, administration of IL-1α during IL-2 therapy in mice decreased cls without affecting therapeutic efficacy (96).

TNFα, IL-1β and IFNγ are capable of PMN activation, which as a result produce proteases and oxygen radicals which also might injure EC. In addition, stimulated PMN activate the complement cascade which in its turn might destroy EC (65).

Due to induction of IL-5 by IL-2 therapy, eosinophils become activated and start to produce the major basic protein. This protein has been found in the perivascular areas of the dermis in skin biopsies of patients undergoing IL-2 therapy and might be responsible for EC damage (35).

Hypotension is a major clinical problem associated with IL-2 therapy. The capillary leak syndrome induces indirectly hypotension, but the direct induction of hypotension by vasodilatation may also occur from nitric oxide production. Nitric oxide is produced by endothelial cells in response to IFNγ in combination with TNFα or IL-1 (97). This

mechanism might also be induced during IL-2 therapy (98,99)

Since TNFα itself might have an important role in anti-tumour activity, the administration of TNFα inhibitors to patients should be approached with some caution. Still, rapid and strong increase of TNFα levels as induced by bolus IL-2 (34) should possibly be avoided to prevent cls, whereas slow and moderate release of TNFα might be beneficial for cell adhesion and migration. This latter situation might be achieved by sc IL-2 therapy, since sc administration of IL-2 induces similar biological effects as bolus and continous iv administration of IL-2 but without concomitant cls. Recently, the use of IL-2 analogues, like R38A and F42K, has been proposed. These analogues have reduced binding capacities to high affinity IL-2 receptors, with the result that they induce less TNFα production than IL-2 but retain the capability to induce cytolytic activity in murine T lymphocytes (100,101). However, the decreased proliferative capacity of lymphocytes in response to these analogues reduces the possible therapeutic value of these analogues.

ADHESION, MIGRATION AND TUMOUR INFILTRATION

As mentioned above, IL-2 therapy induces activation of almost all peripheral blood leucocytes, which, based on results of *in vitro* cytotoxicity experiments, might all play a role in tumour killing. However, *in vivo*, these cells have to cross the endothelial barrier in order to reach the site of the tumour. In addition, for actual tumour killing by cell-cell contact or for local cytokine delivery, they have to infiltrate between the tumour cells. So a prerequisite for successful immunotherapy is the induction of lymphocyte migration to the tumour site. To get there, immune cells have to adhere to and migrate across the endothelium. It is becoming clear that this adhesion and migration is mediated by a cascade of interactions between adhesion molecules on leucocytes and on endothelial cells (reviews in 102-104). Expression and functioning of these adhesion interactions are regulated by many cytokines including TNFα, IL-1, and IFNγ (105).

Influence of IL-2 on adhesion molecules on leucocytes in *vitro*

IL-2 has been shown to induce directly, or indirectly via other cytokines, enhanced expression of CD54, CD58, CD49d, CD11a, CD11b, CD11c, and CD18 on lymphocytes (106-108). In contrast, the expression of L-selectin (involved in lymph node homing of naive cells) was lowered after *in vitro* stimulation with IL-2 (109). NK cells appear to have stored CD11c adhesion molecules in their granules which might explain the important role of CD11c in the rapid IL-2 induced plastic adherence *in vitro* (110) and the rapid disappearance of NK cells *in vivo* during a cycle of IL-2 therapy.

Influence of IL-2 on adhesion molecules on endothelium *in vitro*

Little is known about the direct influence of IL-2 on the expression of adhesion molecules on endothelial cells. IL-2 does not induce ICAM-1 or ELAM-1 expression on cultured endothelial cells (90). The effects of the secondary cytokines, which are induced by IL-2, i.e. TNFα, IL-1 and IFNγ, are better known. These cytokines have various effects on the expression of adhesion molecules (See review in (105)). For instance TNFα and IL-1 induce ELAM-1, VCAM-1, and ICAM-1. IFNγ, which does not induce ELAM-1 and VCAM-1 by itself, is able to induce ICAM-1 and augments the effects of TNFα on ELAM-1 and VCAM-1 expression. IL-4 induces little VCAM-1 expression itself but augments TNFα induced VCAM-1 expression, and suppresses

TNFα and IL-1 induced expression of ELAM-1 and ICAM-1. Many of these cytokine induced expressions, however, are dependent on the type of endothelial cells used (111). The expression of TNFα induced adhesion molecules on endothelial cells follows a time dependent pattern. ELAM-1 is expressed firstly with peak levels after 4-6 hours, followed by a marked decrease. The expression of ICAM-1 and VCAM-1 starts to increase after 4-6 hours with maximum levels after 12 hours, after which ICAM-1 expression remains elevated and VCAM-1 expression slowly declines.

Influence of IL-2 on adhesion and migration *in vitro*

Pankonin *et al.* showed that IL-2 *in vitro* enhances lymphocyte adherence to and migration across rat high endothelial cell layers and that CD11a and CD49d play an important role in these events (112). Adherent lymphocytes mainly consisted of NK and $CD8^+$ T cells. This increased adherence and migration of IL-2 activated lymphocytes appeared to be non-specific for tumour areas, since they showed also increased adherence to arterial endothelium. Similar results were obtained using human LAK cells and human umbilical vein-derived endothelial cells (HUVEC) (113). In addition, it was shown that NK cells adhere better than T cells, whereas T cells, and especially $CD8^+$ T cells have a better capacity to migrate across endothelium than NK cells. Treatment of endothelium with IFNγ markedly increased the migratory capacity of NK cells.

Using a three-dimensional gel of collagen type I as a model for the extracellular matrix (ECM), it could be shown that IL-2 enhances adherence and motility of murine lymphocytes in the ECM (114). NK cells developed higher adherence but less motility than T cells. The motile T cell fraction consisted mainly of $CD8^+$ cells.

Influence of IL-2 on adhesion molecules on leucocytes *in vivo*

Thus far, little is known of the *in vivo* effects of IL-2 on the expression of adhesion molecules on circulating leucocytes.

IL-2 therapy of RCC patients induces increased ICAM-1 expression on NK cells (106) and IL-2 therapy of acute myeloid leukaemia patients induced increased expression of ICAM-1 and LFA-3 on leukaemic blasts (115).

Influence of IL-2 on adhesion molecules on endothelium *in vivo*

Skin biopsies of patients undergoing IL-2 therapy show that IL-2 therapy induces the expression of ICAM-1 and ELAM-1 on the capillary endothelium (90). Since IL-2 is not able to induce ICAM-1 and ELAM-1 expression on cultured endothelial cells, the *in vivo* up-regulation of these adhesion molecules might be due to IL-2 induction of TNFα and IL-1. It has been shown that administration of TNFα to baboons induced rapid and sustained increase of ELAM-1 followed by increases in VCAM-1 and ICAM-1 expression (116). As stated before, simultaneous administration of IL-4 enhanced VCAM-1 expression induced by low concentrations of TNFα. There was a strong correlation between sustained VCAM-1 expression and T cell infiltration. The observed sustained ELAM-1 expression, which is in contrast to the transient expression *in vitro*, might be caused by the induction of secondary cytokines such as IL-1 and IFNγ.

Influence of IL-2 on adhesion and migration *in vivo*

We found in one patient that IL-2 therapy induces enhanced migration of ^{111}In-tropolonate labelled lymphocytes to the primary RCC. In addition, in a rat lung tumour

model, we found that IL-2 administration induces markedly enhanced infiltration of the tumour area by T cells, as compared to rats without treatment (117). Basse *et al.* showed that adoptive transfer of adherent LAK (A-LAK) cells obtained after *in vitro* culture with IL-2 into tumour bearing mice followed by IL-2 administration showed preferential infiltration of A-LAK cells in lung metastases compared to normal lung tissue (118). In addition, resting spleen cells did not infiltrate the metastases. These experiments show that IL-2 induces changes in lymphocytes and probably also in endothelium, causing enhanced tumour infiltration. Preliminary data show that this migration is CD11a/CD54 interaction dependent.

Experiments with infusions of ^{111}In labelled TIL cultured in IL-2 immediately followed by IL-2 therapy demonstrated the preferential localization of TIL at the site of the tumour within 24 hours (28,119). Whether this preferential localization is an intrinsic property of TIL, or due to differences between tumour endothelium and normal epithelium, or a result of IL-2 therapy remains to be determined.

TUMOUR INFILTRATING LYMPHOCYTES

In accordance with the above results, tumour biopsies taken from various types of tumours during IL-2 therapy, as compared to biopsies taken before therapy, show increased leucocyte infiltration into the tumour (120-123). Infiltrating leucocytes appear to be mainly T cells, whereas few macrophages and no NK cells are present. T cells show increased expression of VLA-1, CD25, and HLA-DR. Tumours of responders to IL-2 therapy either expressed HLA-DR already or became HLA-DR positive during treatment (121,123,124).

It is unclear whether the observed increase in lymphocyte numbers present in the tumour during IL-2 therapy is due to enhanced aspecific infiltration by various T cells, by specific infiltration of the tumour, or by proliferation of T cells already present in the tumour.

Fresh TIL display poor cytotoxicity against various targets and show a low proliferative response to IL-2 and CD3 MAb (125-129). Prolonged expansion in IL-2 however induces cytolytic capacity against various tumour targets (127,130). The possible defect in TIL might be analogous to the recently found defect in T cells of tumour bearing mice, that were shown to contain low amounts of CD3γ and to lack the CD3ζ chain completely (131). This protein plays an important role in signal transduction. Another conceivable explanation for the poor TIL activity is local immunosuppression by the tumour. The tumour might produce inhibitory cytokines like TGF-β (132,133). Another way to suppress T cell function is by decreased expression of accessory molecules like B7 and ICAM-1, which play an important role in appropriate induction of T cell activation and cytolytic activity (29,30,134-141). The absence of these molecules, during T cell activation results in T cell anergy (142,143).

Still, TIL cultures do contain T cells which recognize tumour cells in an MHC restricted way. TIL grown in IL-2 produce TNFα, IFNγ, TNFα and GM-CSF in an MHC restricted way when co-cultured with autologous or HLA-matched allogenic tumour cells (144,145). These cytokines might play a role in antitumour activity by increasing the expression of MHC and adhesion molecules on the tumour cells, resulting in an improved tumour recognition by the immune system.

CONCLUSION

This review has given a survey of the immunomodulatory effects of IL-2 in human cancer patients. It is clear that IL-2 induces a broad scala of biological events, which in some cases lead to tumour regression. Despite the enormous amount of data obtained during the last ten years, the mechanism of IL-2 therapy is still not clear.

However, since there is mounting evidence that T cells play an important role in anti-tumour activity, it might be a good idea to focus our attention on these cells. In this respect, three hypothetical events can be put forward that have to occur before IL-2 therapy induces tumour regression.

Firstly, IL-2 therapy activates T cells. This activation might be induced both directly by stimulation via the constitutive IL-2R as well as indirectly by the activation of professional APC via secondary cytokines. Appropriate activation of T cells by IL-2 *in vivo* might only be induced in those cells to which foreign antigens are simultaneously presented. In the absence of this antigen, IL-2 might induce T cell anergy.

Secondly, IL-2 therapy induces (indirectly) activation of the endothelium, and up-regulation of adhesion molecules on T cells, which results in enhanced migration of T cells to the tumour.

Thirdly, IL-2 therapy induces enhanced antigen presentation at the tumour site by induction or enhancement of MHC expression on tumour cells and antigen presenting cells.

All these events must be induced during IL-2 therapy to result in tumour regression.

To gain further insight in the mechanism of IL-2 therapy, more attention should be given to the IL-2 induced events that lead to T cell activation. This includes phenotypical and functional analysis of T cells, longitudinal studies of the cytokine and adhesion cascade, APC activation, and changes in tumour cell phenotype.

Another aspect that needs attention is the induction of toxic side effects. It is clear that TNFα is the central mediator of the toxic side effects of IL-2 therapy. Subcutaneous administration of IL-2 leads to less toxicity, enabling the administration of higher doses of IL-2. Whether the adversary side effects are ultimately exerted by NK cells, polymorph nuclear leucocytes, or nitric oxide, antibodies against TNFα or soluble TNF-receptors might be useful in inhibiting the toxic side effects.

None of the variations on IL-2 therapy thus far, including combinations with other cytokines or with adoptive cell therapy, have led to increased response rates. Even TIL therapy which has been reported to induce higher response rates, appears to be of no real additional value. Improvement of IL-2 therapy should indeed be directed to improvement of T cell activation. This should not only include direct T cell activation, but also the induction of T cell migration, and antigen presentation by APC and tumour cells, establishing the right set of stimuli for optimal T cell activation, leading to optimal immunotherapy. Since IL-2 alone hardly induces GM-CSF production, the combination of IL-2 with GM-CSF might be a very good candidate for inducing biological effects, i.e. stimulation of APC, which might not be induced by IL-2 alone. Recently, it has been demonstrated that B16 murine melanoma cells transfected with GM-CSF DNA induced a much better immune-response, resulting in long-lasting anti-tumour immunity, than tumour cells transfected with other cytokines like IL-2, IL-4, IL-6, TNFα or IFNγ (26). This anti-tumour immunity may result from the effects of GM-CSF on professional APC (27,146). With respect to the formation of better APC, GM-CSF or B7 transfected tumour cells can be used as antigen presenting cells themselves (29,30,135), provided

they present tumour specific antigens in a MHC restricted way. When the tumour cells do not express tumour specific antigens, specificity can be introduced into IL-2 therapy by the administration of bispecific monoclonal antibodies directed against a tumour associated antigen and the CD3 antigen on T cells.

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